

Cooking temperature effects on the forms of iron and levels of several other compounds in beef semitendinosus muscle

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Abstract

The influence of final cooked temperature on the form of iron present and on the concentration of taurine, carnosine, coenzyme Q₁₀ and creatine was investigated in surface and inner parts of 30-mm thick steaks from beef semitendinosus muscle ($n = 6$). The use of a fast, dry-heat cooking method with a Silex clam cooker (set at 200 °C) led to cooking times ranging from 5.6 to 8.6 min for final internal temperatures of 60 and 85 °C, respectively. The proportion of iron as soluble haem iron decreased from 65% in uncooked meat to 22% when cooked to 60 °C and then decreased more gradually with increases in final cooked temperature. The proportion of insoluble haem iron increased in a reciprocal manner, while changes in the proportions of soluble and insoluble non-haem iron were relatively small, but increases in the percentage of insoluble non-haem iron with increasing final temperature were significant ($P < 0.01$). Changes in the forms of iron with cooking generally took place more rapidly in surface samples than inner samples. On a dry-matter basis, concentrations of taurine, carnosine, coenzyme Q₁₀, and creatine all decreased with cooking, but the decreases were greatest for taurine and creatine. Losses of creatine were at least partly due to conversion to creatinine, and, along with the other compounds, probably included some loss in cooking juices. It is concluded that despite these changes with cooking, beef semitendinosus muscle remains a good source of iron and a useful source of the potentially bioactive compounds taurine, carnosine, coenzyme Q₁₀ and creatine.

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1. Introduction

Iron in meat may be categorised according to its solubility in water and whether or not it is in the haem form, with the soluble haem form of iron at the site of absorption being the most likely to be bio-available (Wienk, Marx, & Beynen, 1999). Previous research has shown that cooking affects the proportion of iron in the soluble haem form (Buchowski, Mahoney, Carpenter, & Cornforth, 1988; Kristensen & Purslow, 2001; Purchas, Simcock, Knight, & Wilkinson, 2003). For example, beef semitendinosus muscle cooked within plastic bags in a controlled-temperature water bath contained 72% soluble haem iron in the uncooked state, but after 30 min at 60 °C

this was reduced to 42%, and after 30 min at 80 °C it was less than 5% (Purchas et al., 2003). This method of cooking is not in common use, however, and the extent to which the results apply to meat cooked using a rapid dry-heat method is not clear. The objective of the current work was to evaluate the effects of cooking to several end-point internal temperatures on the form of iron in the surface layers and the inner part of steaks cooked in a clam cooker. Cooking at a set temperature has been shown to affect, not only the form of iron in meat, but also the concentrations of several compounds with potentially bioactive properties including taurine, carnosine, coenzyme Q₁₀, and creatine (Purchas, Rutherford, Pearce, Vather, & Wilkinson, 2004). Therefore, a second objective of the current work was to measure the concentration of these compounds in the same samples of semitendinosus muscle.

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2. Methods and materials

2.1. Muscle samples and cooking procedures

Six bovine semitendinosus muscles were obtained from prime cattle at a commercial meat plant on the day following slaughter. The background of the animals involved were not known in detail, but they were steers or heifers of beef breeds or their crosses that had been grown and finished on pasture. The muscles were stored at 0–3 °C until four days *postmortem* at which time seven 30-mm thick steaks were prepared from each muscle after removing the tapering parts at each end of the muscle. The steaks were trimmed of epimysial connective tissue and squared up so they weighed between 140 and 160 g before being allocated at random to one of the following seven treatments. One steak was sampled directly as the uncooked control and the others were cooked in a clam cooker (Silex Domestic Grill, model 619.80, set at 200 °C) to final internal temperatures of 60, 65, 70, 75, 80, or 85 °C. Internal temperature was determined using Fluke 80PK-5A Type K thermocouples piercing probes attached to a 52K/J Fluke thermometer. After cooking the cooked steaks were placed in plastic bags and held at 0–3 °C for approximately 24 h, at which time the exudates released by the cooked meat into the bag were weighed. Each cooked steak was then separated into an inner and a surface sample of approximately equal size. The surface sample comprised top and bottom slices of approximately one-fifth the thickness of the steak together with 2–3-mm thick slices from the exposed edges. The inner and surface samples were immediately homogenised for two periods of approximately 10 s by chopping in a food processor (Magimix Cuisine 5100, using the 300 ml minibowl). They were then subdivided into two sub-samples for the analysis of iron and the bioactive compounds, respectively, before being frozen at –20 °C.

2.2. Analytical procedures

The bioactive group of compounds were assayed in freeze-dried samples of the chopped, cooked material and the uncooked meat as described by Purchas et al. (2004). Briefly, taurine and carnosine were quantified using an HPLC system after a buffer extract (67 mM sodium citrate buffer, pH 2.2) had been passed through an ultrafilter with a 5000 MW cutoff. Coenzyme Q₁₀ was measured in hexane extracts by HPLC. Creatine and creatinine were assayed spectrophotometrically using enzyme-based systems in an auto-analyser (Purchas et al., 2004).

The proportions of iron as haem iron and non-haem iron in the water soluble and water insoluble fractions of uncooked or cooked meat were assessed as described by Purchas et al. (2003). Briefly, haem iron was assessed

using the colorimetric method of Hornsey (1956), and non-haem iron was assayed colorimetrically using the ferrozine method after removal of haem iron by tri-chloroacetic acid precipitation.

2.3. Statistical analysis

Proportions of the four iron fractions were analysed using a randomised block design with seven treatments (uncooked plus six final internal temperatures) and with muscle as a blocking factor. Differences between pairs of treatments were assessed by least-significant differences within the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The same model was used for the cooked samples only to evaluate differences between inner and surface samples. For the bioactive compounds, which were measured in uncooked samples plus surface and inner samples of steaks cooked to 60 and 80 °C only, a set of orthogonal contrasts was used to evaluate treatment effects as specified in Table 4. Muscle was again included as a blocking or replicate effect.

3. Results and discussion

3.1. Basic characteristics

Characteristics of the steaks and of the cooking process are shown in Table 1. The range of mean uncooked steak weights for the six cooking treatments was less than 10 g, but differences between the six muscles were highly significant ($P < 0.001$). As expected, cooking losses increased at a decreasing rate with increasing final cooked temperature from less than 28% at 60 °C to more than 38% at 85 °C. Such cooking losses should not be extrapolated to other muscles, however, as appreciable muscle differences have been reported under standard cooking conditions (Jeremiah, Dugan, Aalhus, & Gibson, 2003). In that study, for example, the cooking loss for beef semitendinosus muscle (as used here) was about 22% higher than the lumbar longissimus muscle.

Cooking time also increased with increased temperature (Table 1), but even at the highest temperature the mean was only 8.55 min. The amount of exudate released from the steaks after they had been removed from the cooker decreased with increasing final cooked temperature, presumably because more moisture was lost by evaporation during cooking. The data in Table 1 show that samples taken to represent the inner and surface parts of the cooked steaks were of a similar size. The water content of sub-samples of cooked meat (Table 1) showed the expected decrease with increasing cooked temperature, with values for surface samples always being lower than for corresponding inner samples. These values for percentage water are based on the loss in weight with freeze-drying and, based on previous mea-

Table 1

Characteristics of the steaks from beef semitendinosus muscle that were cooked to the six final internal temperatures shown ($n = 6$)^a

	Final internal temperature (°C)							Replicate effect ^b	<i>R</i> ² (%), RSD
	60	65	70	75	80	85	Effect ^b		
Uncooked steak weight (g)	151.2	149.8	146.5	149.1	142.5	144.0	ns	***	68, 10.6
Cooking loss (%)	27.2a	30.2b	33.5c	35.4cd	37.2de	38.7e	***	*	90, 1.7
Cooked meat exudate (%)	4.30c	5.42c	4.31bc	3.51b	3.60b	1.85a	***	ns	59, 1.18
Cooking time (min)	5.63a	6.10a	6.90b	7.63b	8.47c	8.55c	***	***	87, 0.62
Sub-sample size (% of cooked weight)									
Surface sample	47.0	48.7	47.2	46.3	48.7	47.6	ns	ns	44, 2.1
Inner sample	53.0	51.3	52.8	53.7	51.3	52.4	ns	ns	44, 2.1
Water content of cooked meat (%)									
Surface sample	62.3d	62.0d	60.3c	58.8b	58.2b	56.9a	*	***	90, 0.84
Inner sample	68.1e	67.1e	65.6d	64.0c	62.7b	61.5a	*	***	90, 0.97
Iron content of cooked meat (µg g ⁻¹)									
Surface sample	28.4	28.3	28.1	29.9	27.6	31.9	ns	***	92, 2.47
Inner sample	21.5	21.8	23.1	24.3	24.6	25.3	ns	***	65, 2.47
Iron recovery (%) ^c	99.6	96.0	93.7	95.9	90.7	98.3	ns	***	90, 8.3

^a Measures of goodness-of-fit of the statistical model are given by coefficients of determination (R^2 , %) and residual standard deviations (RSDs). Means within a row do not differ significantly ($P > 0.05$) if they have a common letter or if they have no letters.

^b ns, $P > 0.05$; *, $P < 0.05$; ***, $P < 0.001$.

^c Iron in the surface plus inner portions as a percentage of iron in the initial uncooked sample. The iron concentration in the uncooked sample was $16.4 \mu\text{g g}^{-1} \pm 0.46$ (mean \pm SE).

surements, are likely to be about one percentage point lower than water percentage based on oven drying.

Iron concentrations were consistently higher for the surface samples because they contained less water, even though loss of iron into cooking juices would have been more likely from those samples. Temperature effects on the levels of iron were not significant for either the surface or the inner samples. The percentage of total iron recovered in the inner plus surface samples was not affected by temperature either (Table 1). Recovery levels were generally greater than 90% indicating that iron losses during cooking were low (<10%) compared with values of up to 20% reported for a slower water-bath cooking method (Purchas et al., 2003). It was not possible to calculate recovery values separately for the inner and surface samples because it was not known how much of the cooking losses to attribute to those two sources.

3.2. Proportions of iron in four fractions

Changes in the proportion of iron in the four fractions for the uncooked meat and for the surface and inner samples of cooked meat are shown in Fig. 1. Levels of significance for treatment and replicate effects are given in Table 2, while treatment and replicate effects on the differences between inner and surface samples for iron levels within each steak are given in Table 3. Results in Fig. 1 show that for both the inner and surface samples the general patterns of changes were for percentage soluble haem iron to decrease markedly from the uncooked state when it was about 65%, to the

cooked state when for a final temperature of 60 °C it was about 22%. As cooking temperature increased the percentage of soluble haem iron after cooking decreased, with a level of about 4% at 85 °C. These decreases were approximately matched by increases in the proportion of insoluble haem iron, presumably due to heat denaturation of the myoglobin molecule (Renerre, 1990). The two fractions of non-haem iron showed a similar pattern to the haem iron with the soluble fraction decreasing and the insoluble fraction increasing at higher temperatures. These patterns are generally similar to those reported by Purchas et al. (2003) for the same muscle when steaks were cooked using a slower more closely controlled method in which the steaks were inside plastic bags that were suspended in a water bath at specific temperatures for periods of 30 or 90 min. These were much longer cooking times, however, than those involved in the current work, which averaged less than 9 min even at the highest temperature (Table 1).

Although the general patterns for the inner and surface samples with increasing temperature were similar (Fig. 1), there were numerous instances of where the difference (inner value minus surface value) was significantly different from zero (Table 3). For soluble haem iron this was particularly the case at the lower temperatures where levels were significantly higher for the inner samples (Fig. 1) presumably due to inner samples being at a lower final temperature than the surface samples. For insoluble haem iron the differences were smaller with values at 60 and 65 °C being lower or not significantly different for inner samples, but at 70–85 °C levels for

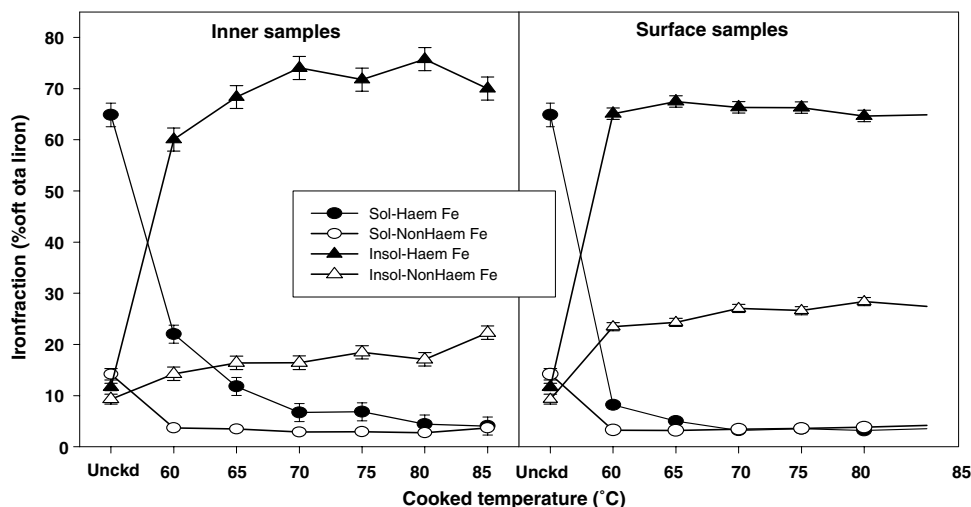


Fig. 1. The percentage of iron in four fractions (means \pm SE) within beef semitendinosus muscle in the uncooked state (Unckd) and after cooking to final internal temperatures of from 60 to 85 °C.

Table 2

Levels of significance for the effects of increasing cooking temperature from 60 to 85 °C (means \pm SE are given in Fig. 1) and for replicate effects ($n = 6$) for the percentage of iron in four forms for surface and inner samples from beef semitendinosus steaks^a

	Cooking temperature effect ^b	Replicate effect ^b	R^2 (%), RSD
Surface sample (% of total iron in the sample)			
Soluble haem iron	***	ns	74, 1.32
Soluble non-haem iron	ns	*	51, 0.62
Insoluble haem iron	ns	***	68, 2.71
Insoluble non-haem iron	**	***	80, 1.94
Inner sample (% of total iron in the sample)			
Soluble haem iron	***	ns	77, 4.31
Soluble non-haem iron	*	***	78, 0.56
Insoluble haem iron	***	***	71, 5.52
Insoluble non-haem iron	**	***	75, 3.21

^a Measures of goodness-of-fit of the statistical model are given by coefficients of determination (R^2 , %) and residual standard deviations (RSDs).

^b ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 3

Differences between inner and surface samples for the percentage of total iron in four fractions for beef semitendinosus muscles cooked to final internal temperatures of from 60 to 85 °C^a

	Final internal temperature (°C)							Replicate effect ^b	<i>R</i> ² (%), RSD
	60	65	70	75	80	85	Effect ^b		
Difference (inner sample–surface sample) (% of total iron)									
Soluble haem iron	13.84***	6.77***	3.46*	3.31*	1.25ns	0.49ns	***	ns	75, 3.37
Soluble non-haem iron	0.44ns	0.27ns	−0.54ns	−0.64ns	−1.07**	−0.48ns	ns	**	57, 0.90
Insoluble haem iron	−5.07*	0.87ns	7.70**	5.48*	11.10***	5.14*	***	**	69, 5.24
Insoluble non-haem iron	−9.21***	−7.92***	−10.62***	−8.15***	−11.28***	−5.15**	ns	**	59, 3.55

^a Measures of goodness-of-fit of the statistical model are given by coefficients of determination (R^2 , %) and residual standard deviations (RSDs). The significance with which each mean difference differed from zero is shown.

^b ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

inner samples were significantly higher. These higher values in terms of a percentage of total iron are likely to indicate that more haem iron was converted to non-haem

iron at the higher temperatures for the surface samples, a suggestion that is supported by the higher levels of insoluble non-haem iron in the surface samples (Fig. 1,

Table 3). Schricker and Miller (1983) also reported higher levels of non-haem iron in surface samples of cooked meat, but they did not make a distinction between soluble and insoluble non-haem iron. The percentage of soluble non-haem iron showed only small differences between the inner and surface samples (Table 3).

Replicate effects were not significant for soluble haem iron percentage but were for the other three fractions (Tables 2 and 3) indicating that the differences between the six muscles for those three fractions tended to be consistent across the uncooked sample and all the five cooking-temperature treatments.

3.3. Concentrations of taurine, carnosine, coenzyme Q₁₀, creatine, and creatinine

Results in Table 4 show that concentrations of all five of these compounds on a dry-matter basis differed significantly between the cooked and uncooked samples, although in the case of carnosine the differences were relatively small, particularly for the inner samples. Cooking brought about a decrease in the concentration of each of the compounds except creatinine, which increased to about ten times the uncooked concentration in the cooked samples, presumably due to the conversion of creatine to creatinine (Macy, Naumann, & Bailey, 1970).

For taurine and coenzyme Q₁₀ concentrations, there were clear decreases from the uncooked to the cooked state, but differences between final temperatures of 60 and 80 °C, and between inner and surface samples at either of these temperatures were not significant (Table 4). For coenzyme Q₁₀ this contrasts with results obtained for two lamb muscles cooked to 70 °C (Purchas et al., 2004) where concentrations on a dry weight basis were higher in meat cooked for 90 min within a plastic bag in a water bath. Possibly the longer, slower cooking was responsible for this difference. The reduction by about 40% in taurine levels with cooking was similar to

that reported for lamb meat (Purchas et al., 2004) and was probably due at least in part to losses of this water-soluble compound in cooking juices. Carnosine is also water-soluble, but its losses were appreciably less than for taurine and were significantly lower for inner samples than for surface samples (Table 4).

Decreases in water-soluble creatine were greater for surface samples than inner samples in a similar pattern to carnosine, but, unlike taurine, carnosine and coenzyme Q₁₀, the loss of creatine was significantly higher for samples cooked to 80 °C than 60 °C, probably due to a greater conversion to creatinine (Harris, Lowe, Warnes, & Orme, 1997; Macy et al., 1970). Creatinine levels showed an 8-fold increase from uncooked to cooked to 60 °C, but an 11-fold increase when cooked to 80 °C. Conversely, creatine levels decreased to 65% and 55% of the uncooked level when cooked to 60 and 80 °C, respectively. Creatinine levels, however, were not different between the surface and inner samples (Table 4) suggesting that the greater loss of creatine from surface samples was due to higher losses into cooking juices. Increases in creatinine levels did not fully account for decreases in creatine levels. For example, cooking to an internal temperature of 80 °C resulted in the creatine concentration decreasing by 844 mg (100 g DM)⁻¹, but the increase in creatinine was only 37% of that amount.

The replicate effect was significant for all five of the compounds measured (Table 4), indicating that those muscles with higher concentrations in the uncooked meat also tended to have higher concentrations following cooking. There was a positive association between concentrations of taurine and coenzyme Q₁₀ (simple correlation (r) = 0.83 for uncooked samples), and negative correlations between carnosine levels and the levels of both coenzyme Q₁₀ and taurine (r = -0.71 and -0.77, respectively, for uncooked samples). These results are generally consistent with the between-muscle and between-tissue comparisons reported previously (Purchas et al., 2004).

Table 4

The effects of final internal temperature, position within the steak, and replicate ($n = 6$) on concentrations (mg (100 g DM)⁻¹) of taurine, carnosine, coenzyme Q₁₀, creatine and creatinine in beef semitendinosus muscle expressed on a dry-matter (DM) basis^a

	Uncooked sample	60 °C		80 °C		Contrast effect ^b				Replicate effect ^b	R^2 (%), RSD
		Surface	Inner	Surface	Inner	U vs Rest	60 vs 80	S vs I in 60	S vs I in 80		
Taurine	132.5	77.9	97.6	65.5	80.5	***	ns	ns	ns	***	88, 24.9
Carnosine	1531	1257	1521	1266	1450	*	ns	**	*	**	71, 133
Coenzyme Q ₁₀	6.91	5.27	4.60	5.13	4.39	***	ns	ns	ns	***	91, 0.75
Creatine	1961	1132	1430	947	1117	***	***	***	**	***	97, 76
Creatinine	29.4	222.1	250.5	318.5	339.6	***	***	ns	ns	*	96, 29.2

^a Measures of goodness-of-fit of the statistical model are given by coefficients of determination (R^2 , %) and residual standard deviations (RSDs). The four orthogonal contrasts were: (1) uncooked versus the other four treatments (U vs Rest), (2) a cooking temperature of 60 versus 80 °C (60 vs 80), (3) surface samples versus inner samples cooked to 60 °C (S vs I in 60), and (4) surface samples versus inner samples cooked to 80 °C (S vs I in 80).

^b ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

3.4. General discussion and conclusions

A primary objective of this study was to determine whether the changes in the form of iron and in the concentrations of selected bioactive compounds that occur during cooking within plastic bags in a temperature-controlled water bath were similar to those occurring when cooking was by means of a fast, dry-heat method. The contrast in cooking time is illustrated by the fact that for the highest final internal temperature group (85 °C), a mean cooking time of only 8.55 min was required, while for the water bath methods employed by Purchas et al. (2003) periods of 30 or 90 min were used. For both cooking methods the largest change was a decrease in the proportion of soluble haem iron and a corresponding increase in insoluble haem iron, presumably as a result of myoglobin being denatured. To investigate this change in more detail, temperatures of less than 60 °C and/or cooking times of less than 5.6 min will need to be used. The patterns of decrease in soluble haem iron are consistent with results of studies, such as that of Lytras, Geileskey, King, and Ledward (1999), that have shown that the rate of denaturation and insolubilisation of myoglobin in meat increases markedly with increasing temperature over the range from 55 to 75 °C, and that at any set temperature it initially follows first order kinetics.

The relative proportions of haem and non-haem iron reported here are similar to those reported previously (Purchas et al., 2003), with the non-haem iron making up from 20% to 30% of total iron following cooking (Fig. 1). This is a lower value than that reported by Kristensen and Purslow (2001) where non-haem iron in ground pork cooked at 80 °C was over 60% of the total iron. This is probably because haem iron levels are lower in pork (about 58% in their study versus 75% in the current work with beef), and also because the pork was cooked for 2 h and the conversion of haem iron to non-haem iron is dependent on the time of heating as well as the temperature (Chen, Pearson, Gray, Fooladi, & Ku, 1984; Jansuittivechakul, Mahoney, Cornforth, Hendricks, & Kangsadalampai, 1985). Jansuittivechakul et al. (1985), for example, reported that boiling of ground beef led to decreases in the percentage of haem iron from 53% of total iron after 2 min to 43% after 30 min and 38% after 90 min. Differences in the form of iron between surface and inner samples were generally smaller than changes with increasing temperature. Further information on changes in the ratio of haem iron to non-haem iron with cooking was provided by Buchowski et al. (1988) and Han et al. (1993).

In terms of iron bioavailability, decreases would be expected with cooking both because of the decrease in the proportion of total iron as haem iron, as well as the decrease in iron solubility. Of these two effects, it is likely that a lower proportion of haem iron is potentially

more important (Conrad & Umbreit, 2000; Uzel & Conrad, 1998; Wienk et al., 1999), but this was a relatively small change with the decrease in total haem iron being only 1.8 and 7.4 percentage points for the 85 °C treatment group relative to the uncooked samples for inner and surface samples, respectively (Fig. 1). In contrast, the decrease in total soluble iron for the 85 °C treatment group relative to the uncooked meat was 70.6 percentage points for both the inner and surface samples (Fig. 1). This loss of solubility, however, was for the meat in the form in which it would be consumed rather than in the product at the site of iron absorption in the duodenum. It is likely that further changes in iron solubility will occur as digestion takes place in the stomach and small intestine (Conrad & Umbreit, 2000).

The cooking treatment used in this study led to significant declines in all the compounds with potential bioactive studies, with the declines being greater for taurine and creatine than for carnosine and coenzyme Q₁₀. Losses of carnosine and creatine were higher in surface samples than in inner sample, but it was not possible to determine the extent to which this was due to losses in exudates from the steaks or to breakdown of these compounds as a result of the higher temperatures.

The quantities of these compounds that are required to be of value in the human diet have not been defined closely so it is not possible to accurately evaluate the importance of the changes reported here. Although their levels in a 100 g serving of cooked meat are appreciably lower than those widely available as commercial supplements (discussed by Purchas et al., 2004), meat remains a significant source of all four compounds. The extent to which they will provide clear benefits to individual subjects awaits the outcomes of further research in this area. Additional information is also needed on the bioavailability of these compounds from dietary meat relative to other sources.

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